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FROM THE EDITOR

According to a recent National Science Foundation article, although the United States “...influence on world science and technology remains strong...the number of U.S. science and engineering articles in major peer-reviewed journals flattened in the 1990’s, after more than two decades in growth.” Meanwhile, academic development around the globe—in China, Japan, Singapore, South Korea, Taiwan, and the European Union—is growing rapidly, outpacing the US in the past two decades. While there is no immediate cause for concern, it is surprising, given the speed with which science has been advancing in recent years. As new technology and new techniques for research are being discovered, we should expect scientific innovation to be on the rise. More importantly, scientific advancement has become the livelihood of our modern culture, creating a need for its continued expansion. We depend on it for better medicine, for safer and more efficient technology, and most of all, we look to science for solutions to the numerous worldwide crises we face, like global warming, depletion of fuel resources, and environmental degradation. Therefore, it is imperative that our generation provide the impetus to continue this legacy of scientific progress, so as to prevent it from stagnating when we are most in need of it.

The Caltech Undergraduate Research Journal gives students’ research a way to reach the scientific world. We provide a way for young and budding researchers, who may not otherwise have the opportunity to publish their research despite its potential benefit for science, to introduce their ideas and their findings to the scientific community. There is nothing holding back these young scientists from conducting meaningful research for the good of mankind; personal resolve to endow knowledge upon the rest of the world drives them, and we supply the means to do so.

Whether they are amateur or professionals, young or old, and though they come from different cultures and different backgrounds, there is one thing that all scientists share; a deeply imbedded passion for observing, understanding, and changing the world around them into a better place. It is this genuine enthusiasm and zeal of the students that sit at the soul of the Caltech Undergraduate Research Journal. We want the world to know about the accomplishments of these forthcoming scientists who have begun to dedicate their lives to the advancement of the scientific community. After all, these bright young minds are the hope for our future.

Regards,

Helen Lee
Editor-in-Chief

How did you first become interested in Math?

My parents gave me a roulette wheel when I was seven or eight. The notion of these random results intrigued me greatly. Of course, I had no concept of any sort of mathematics, probability and statistics included, back then, but the idea that chance governed these results fascinated me. At my high school, there was little stimulation from mathematical coursework, and my teachers were not very good. During my freshman year at Caltech, I took Math 1 with Tom Apostol, which was wonderful. Gradually, I began to realize I was both good at math and liked it. However, I never really looked at research in math as a career until probably my junior year at Caltech. At this time, I wrote what became my first published paper over Christmas Break. That was when I started to think I could make a career out of math.

What led you to Statistics and Probability?

Initially, I was interested in Combinatorics and Group Theory in addition to Statistics and Probability. I went to Graduate School in the math department at Cornell, where I realized I wanted to do my thesis work in Statistics and Probability. After my Ph.D. work at Cornell I got a job in the math department at Northwestern. And after talking to some of my former professors in the math department at Caltech (which is too small to have its own Statistics/Probability Department), I was invited back as a faculty member. I often joke that I’ve been at Caltech since I was 17, with six years off for good behavior.
What kind of consulting projects do you take part in?

I used to consult for JPL on a weekly basis, mostly doing probability models to optimize certain tradeoffs—what’s called “operations research.” I enjoy fundamental math, so I tend to avoid looking at massive data sets, but I’ve done a great variety of statistical consulting with people on and off campus over the last forty years, trying to devise good methods for them to use in analyzing data and making decisions. In recent years I’ve done a lot of work as a statistical expert in litigation and regulatory issues, which is fun and challenging.

Is there a specific project you’ve found particularly interesting?

One early project I worked on at JPL involved receiving data from deep space probes. To summarize the problem, there are certain rates of failure for the equipment in the Deep Space Network that receives the signals at mission-critical times. I was asked to develop a statistical method that would monitor the data stream of random failures over time and react quickly if the rate of failures ever significantly increased, which would require action. That led me to work on how to formulate and propose solutions for what turned out to be a fundamental type of mathematical statistics problem, now called a “change point” problem. Such problems occur in a variety of applications—to quality control in industry, signal detection, medical and epidemiological applications, and so on. There have been a lot of good statistics papers published about this problem over the last forty years and I’ve continued to do research on it.

How do you approach teaching students at Caltech?

I currently teach 21.11, an introduction to Probability and Statistics, and 21.112, a more advanced course on the same subject. I primarily try to show my students some general ideas that will enable them to make useful applications of statistics and probability. I want my students to be able to think independently and to be able to formulate their own analyses for problems rather than rely on “cookbook” methods and automated statistics packages.

As a Caltech Ahm, do you have any advice for undergraduates at Caltech?

The most important piece of advice I have is don’t let Caltech get you down and don’t underestimate yourself. Whichever we go and whatever work or further study we do, most of us start to feel much smarter soon after graduating.
Combinatorial Optimization
over Maximal Intersecting of Subsets

student: David Renshaw
mentor: Cheng-Yeaw Ku

One of the most basic structures in mathematics is that of a finite set and its subsets. Proving theorems without it is like baking cakes without a mixing bowl—impossible because there is nowhere to put anything. Therefore it is no surprise that combinatorialists, who specialize in pointed questions about elementary structures, have often studied collections (affectionately called “families”) of subsets satisfying various criteria. One particularly natural question, however, has remained unsolved for more than twenty years. Roughly it asks whether a certain optimization problem concerning families of intersecting subsets of a given finite set has always a simple solution. An affirmative answer would imply the resolution of another, open question that had stumped even the legendary mathematician Paul Erdős. The present paper documents an attempt to use one specific idea—what we will call “reductive smoothing” to attack this problem. Although the method apparently leads to a dead end, it yields some insights along the way that are, perhaps, interesting in their own right.

An Intuitive Conjecture

The problem itself is easily stated. The only notion that requires special definition is that of a “maximal intersecting family” of subsets of $S$. Here, “intersecting” means that any two members of such a family share at least one common element (not necessarily the same for all pairs) and “maximal” means that if the family adopts any new member it will cease to be intersecting. One important fact about these families is that each consists of exactly half of the subsets of $S$. Now suppose that every subset of $S$ is assigned a weight, with the restriction that each subset must not weigh more than any of its subsets. That is, suppose weight is non-increasing with respect to inclusion. (The earlier question alluded to above assumes furthermore that weight can only take the values 0 or 1.) Then suppose that we would like to find a maximal intersecting family whose total weight is as large as possible. In 1979, Daniel Kleitman conjectured that the only families we need to check are those which contain a singleton—that is, a set of one element. The aim of our research was to prove this conjecture.

Intuitively, the validity of the conjecture might seem almost maddeningly obvious. Families that contain a singleton are in a sense “pushed down” as far as they will go (the figures provide an explanation of this terminology). Since smaller subsets are heavier, why should not the heaviest family contain as many of them as possible? To put it another way, imagine a maximal intersecting family that does not contain a singleton but has maximum weight. These will be some subset $A$ that is a member but that contains no other member. We can obtain another maximal intersecting family by trading $A$ for its complement $A'$, so the weight of $A$ must be greater than the weight of $A'$. But then should we not be able to make a bunch of favorable trades to include all of those heavy subsets contained in $A'$? Or if heavy members somewhere else prevent us from doing this, should we not be able to make favorable trades to include their subsets? In either case, we will ultimately be led to a family that contains a singleton. Making this line of reasoning rigorous, however, is not easy.

A Smooth Approach

Fortunately, we do not need to consider every possible weight function individually. A technique introduced by Kleitman and later elaborated upon by Peter Fishburn allows us instead to focus our attention on the maximal intersecting families themselves. The idea, which we will call “smoothing,” is to take an arbitrary family and show that it can never be lighter than some average of singleton-containing families. When smoothing succeeds, it demonstrates that one of the singleton families must weigh at least as much as the original family. Hence, if we can prove that every maximal intersecting family can be smoothed, then we will have proved the conjecture. In fact, if we ever find a family that cannot be smoothed then we will actually have disproved the conjecture.
This equivalence (which we will not prove here) is encouraging. The smoothing process can be illustrated by an example. Suppose that \( F \) is a maximal intersecting family which contains the doubling \((a,b)\). Each member of \( F \) will then fall into one of three disjoint classes, depending on whether it contains \( a \), \( b \), or both. Let \( x_a \) and \( x_b \) be the respective sizes of these classes. We know right away that \( x_a \geq 2^{x_b} \) because that is how many supersets \((a,b)\) has, and all of them must be in \( F \) by maximality. This leaves another \( 2^{x_b} - 2 \) members of \( F \) that are split in some way between the other classes. It turns out that the total weight of \( F \) must be less than or equal to \( 2^{x_b} \) times the weight of the family containing the singleton \( (a) \), plus \( 2^{x_b} - 2 \) times the weight of the family containing \((b)\). To see why this is so, consider the following interpretation. Imagine that we have written down all of the subsets of our ground set, and we place a one-ounce ball of clay on each subset that is a member of \( F \). To calculate the weight of this configuration we multiply the real weight of each ball of clay by the value of the weight function on the subset it covers. Now, whenever we transfer clay from some subset to a subset contained in it, the weight of the configuration will remain constant or increase. In fact, it is not too difficult to show that we can always transfer clay in this favorable way so as to evenly distribute the \( x_a \)-class-ounces of clay among all subsets that contain a but not \( b \), and the \( x_b \)-class-ounces among all subsets that contain \( b \) but not \( a \). Furthermore, each of the \( x_a \) balls still intact after this can be split into an \( x_b/2 \)-ounce portion and a \( x_b/2 \)-ounce portion. It is now clear that the weight of \( F \) is no greater than the weight of an average of singleton families.

Our idea is to generalize this technique. Evidently, smoothing succeeds when \( F \) has a doubling because then we can forget about all but two singleton families. It would be helpful in the general case if we could forget even about just one singleton family. There turns our to be a nice property which, when exhibited by a family, guarantees that we can indeed do this. For any maximal intersecting family \( F \) and any element \( x \) of the ground set \( S \), we may consider the induced family \( F^x \) obtained by deleting every instance of \( x \) from the members of \( F \). Of course, \( F^x \) is not necessarily a maximal intersecting family, and in general it is not even intersecting. However, if the members of \( F^x \) can be partitioned into two classes, each of which is intersecting, then the problem of smoothing \( F \) reduces to the problem of smoothing two families on the smaller ground set \( S \setminus \{x\} \).

In other words, by forgetting about the singleton family containing \( x \), we can reduce to a smaller problem and hence allow for an inductive proof of the conjecture.

**Our Optimistic Hypothesis**

Which maximal intersecting families allow such “reductive smoothing”? Clearly, if they all do, then a proof of the conjecture follows immediately. Unfortunately, the Fano plane dashes any such hopes. It is a family of three-element subsets of a seven-element set such that any two members intersect in exactly one element, and each element is contained in exactly three members. It fails the test because any family induced from it by deleting an element necessarily contains a three disjoint members. Nevertheless, the Fano plane poses us little difficulty because the unique maximal intersecting family generated by it can be evenly smoothed. If we place a ball of clay on each member of the Fano plane and on each superset of these members, we can then favorably transfer clay to obtain the configuration where each subset is covered by an amount of clay proportional to its size.

We conjectured that this will always be so: for every maximal intersecting family, either reductive smoothing or even smoothing will succeed. The observation here is that a family \( F \) which cannot be reductively smoothed is forced to have a certain degree of regularity. Namely, for each \( x \) in \( S \), there must be a cycle of odd length that consists of members of \( F \) where adjacent elements intersect only in \( x \). Contrapositively, families that cannot be evenly smoothed seem unlikely to have such a cycle. Consider the case (which will be our focus for the rest of the paper) when \( S \) has an even number \( n \) of elements and \( F \) has no member with less than \( n/2 \) elements. In this situation \( F \) is “pushed up” as far as possible, and thus can probably be smoothed evenly. One exception is when there is an element \( x \) that is not in any size-\( n/2 \) member of \( F \). Then there are exactly as many non-members containing \( x \) as members containing \( x \). But the \( x \)-containing members are the only allowable sources of clay for these non-members, and it turns out they cannot provide quite enough for an even smoothing. However, this family \( F \) is precisely the easiest family to reductively smooth, because \( F^x \) is indeed intersecting. So perhaps the two kinds of smoothing take care of every family in this situation.

**How Necklaces Throw in a Wrench**

Surprisingly, our weighty thinking meets with a counterexample even in this simplified setting. Since a “pushed-up” \( F \) contains no subsets of size less than \( n/2 \), all subsets of size greater than \( n/2 \) are members. This means that our only freedom lies in choosing which size-\( n/2 \) subsets to include in \( F \). The key to our following construction is that we can choose a small number of these to prevent reductive smoothing, and we can choose the rest so as not to contain some element \( x \) which will still prevent even smoothing. Imagine that we have a string and \( n \) beads labeled as elements of \( S \). We choose one bead to set aside and we string up the rest and tie a loop to make a necklace. There are \( n-1 \) ways to choose \( n/2 \) alternating beads on the necklace. Let each of these ways along with the unstrung bead \( x \) defines a member of \( F \). This guarantees that \( F^x \) cannot be partitioned into two intersecting families. We now remove the necklace and proceed to set aside a different bead. The time we have to be careful when stringing up the necklace because some orderings will define subsets that are disjoint from those we have already added as members of \( F \). But it turns out that if \( x \) is large enough then we can always find a good way to do it, and we can in fact continue until we have set aside each bead. The reason

**The Fano Plane:** \( \{a, b, e \}, \{a, d, f \}, \{a, c, g \}, \{b, d, g \}, \{c, d, e \}, \{b, c, f \}, \{e, f, g \} \)
An explanation of ‘pushed up’ and ‘pushed down.’

Subsets of size:


A ‘pushed up’ family for n=4. We hypothesized that any such family would either be evenly or reductively smoothable.

A singleton family for n=6. Such a family is ‘pushed down’ as far as possible.

It is that although each subset we add to $F$ eliminates a number of feasible necklaces; the total number of ways to make a necklace out of $n-1$ beads is large enough to make this irrelevant. When we are finished, we will have chosen $n-1$ or less members of $F$ that together prevent a reductive smoothing. As mentioned before, we choose the rest of the members of $F$ so as not to contain $x$. Now, if $F$ contained no size-$n/2$ subset containing $x$ and we tried to evenly smooth, the total clay deficit for the nonmembers containing $x$ would be (as it turns out) exactly $2^{n/2}$ ounces. Each size-$n/2$ member of $F$ that does contain $x$ can only help this deficit by one ounce. As long as we have chosen $n$ large enough so that $2^{n/2} - n > 0$, it is possible, we find that $F$ cannot be evenly smoothed. Since it also cannot be reductively smoothed, $F$ is our counterexample.

Thus, our approach evidently fails to provide a breakthrough. Reductive smoothing is not strong enough to take care of all families that cannot be evenly smoothed. Does this mean that the technique is worthless? Probably not. Perhaps there is some nice property other than “evenly smoothable” that can be used to fill the gap left by reductive smoothing. Or perhaps we did not define reductive smoothing itself in the most general way. In fact, there do exist non-intersecting families of size $2^n$ that are nonetheless smoothable.

This could possibly be taken into account somehow. In any case, the study of reductive smoothing isolates some of the difficulties inherent in the effort to prove Kleitman’s conjecture. And that is the first step towards overcoming them.

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Further Reading

Red sky at night, sailor’s delight, red sky in morning, sailor’s warning... “cautioned seamen in days of old while casting wary glances at the sky. Even if they did not understand the science behind it, every sailor knew the weather lore because, like other lore, it had withstood the test of time: a red sky at sunset foreshadowed clear weather and fair sailing, but a red sky at sunrise was the harbinger of menacing storms and waves. But although this was once the accepted standard for weather forecast, we have since developed sophisticated methods in meteorology for a more reliable prediction.

For millennia, people have been obsessed with the weather because of its inherent importance in agricultural and seafaring societies, but weather has always been a difficult and temperamental entity; the Greeks even bestowed Zeus, the volatile ruler of gods, with the power of thunder and associated elements of weather to underline his supremacy. But while taming the heavens is beyond human ability, people have long studied weather patterns in an attempt to forecast the weather. Careful observations have been recorded as early as 650 BC by the Babylonians and 340 BC by Aristotle and eventually led to the practice of weather forecasting.

The science of meteorology matured greatly in the 20th century with the advancement of technology. While forecasts once relied solely on local weather station data, the incorporation of mathematical models of the atmosphere through a technique called numerical weather prediction (NWP) was introduced in the 1920s. The modeling requires manipulation of huge datasets and completion of complex computations. The precision required to make these results useful required the development of some of the most powerful supercomputers in the world.

Currently, meteorology relies on satellites to collect global data. The data is then assimilated with the forecast predicted by a numerical model. With computer simulations using physics and fluid dynamics, the state of the atmosphere can be evolved and technologically enhanced for the televised weather forecast on the morning news. However, the complexity of climatic interactions, along with the discovery of new atmospheric processes, makes accurate weather forecasting extremely difficult.
DYNAMICS OF THE ATMOSPHERE

The atmosphere surrounding Earth is a dynamic system involving complex interactions between its various components. It is governed by a delicate feedback system that drastically influences the global climate by amplifying small changes in cloud cover, aerosol concentration, and latent heating.

Decades of close observation and study have revealed well-established cycles that consistently dominate the energy flow of the Earth. Solar heating occurs mostly in the tropics, much of which is covered by ocean. Given the high heat capacity of water, a considerable amount of solar energy is stored in equatorial waters. Oceanic surface currents heading for the arctic poles, such as the Atlantic Gulf Stream and the Pacific Kuroshio current, transport portions of the heat away from the tropics to influence the climate at mid-latitudes.

Upon cooling in the higher latitudes, moisture in the saturated air condenses to produce rain; the solar energy used in the initial evaporation process is then released into the atmosphere as thermal energy. The rate of thermal energy release for each millimeter of rainfall is three times as great as the solar energy that falls on the corresponding surface area. Consequently, precipitation concentrates heat from the tropics by as much as a factor of a hundred in wetter regions. Furthermore, solar heating takes place mainly at the earth’s surface whereas the energy released by condensation occurs at high altitudes. The large amount of heat released with precipitation has a great impact on the global atmospheric circulation.

Through their influence on the exchange of solar and thermal energy, clouds dominate the energy budget of Earth and hence significantly influence global weather. Even small changes in cloud abundance or distribution could alter the climate more than the anticipated changes in greenhouse gases, anthropogenic aerosols, or other factors associated with global climate change. These modifications in turn can give rise to further extreme changes in cloud coverage, creating a vicious cloud-climate feedback system. Meteorologists now believe that the main uncertainties in climate model simulations are due to difficulties in adequately representing the thermodynamic and radiative properties of clouds.

ACCUMULATION OF CLIMATIC DATA

Several meteorological projects are striving to obtain data about the current state of the atmosphere and to integrate new discoveries with historical records. The Reanalysis Project is an investigative study jointly supported by the National Center for Environmental Prediction (NCEP) and the National Center for Atmospheric Research (NCAR). Its goal is to compile new atmospheric analyses using historical data from 1948 and onwards as well as to produce analyses of the current atmospheric state using modeling techniques.

Another project is the Total Ozone Mapping Spectrometer (TOMS), which is a satellite instrument for measuring ozone values. Of the five TOMS instruments that were constructed, four successfully entered orbits. In fact, the Nimbus-7 and Meteor-3 satellites provided global measurements of total column ozone on a daily basis to compile a complete data set of daily ozone from November 1978 to December 1994. Two other instruments have been launched since 1996, and the Earth Probe TOMS continues to collect data.

TOMS consists of an optical sensor capable of measuring the albedo of the earth’s atmosphere, or the amount of radiation reflected back into space, at six narrow spectral bands. Because the total ozone content is proportional to changes of solar radiation near ultraviolet wavelengths, the spatial distribution of the ozone composition of the atmosphere can be inferred by observing several wavelengths. In addition, the TOMS observation data can be used to make quantitative estimates of aerosols such as microscopic dust and smoke.

The primary objective of TOMS is to collect continuous data on global ozone and aerosol content. It has been used increasingly to understand the behavior of aerosol particles in the atmosphere. Capable of observing particles even as they cross the land and sea boundary, TOMS permits the tracking of a wide range of phenomena such as desert dust storms, forest fires, and biomass burning.

MADDEN–JULIAN OSCILLATION

One atmospheric phenomenon which may be newly characterized with TOMS data is the Madden–Julian Oscillation (MJO), an equatorial traveling pattern of anomalous rainfall. In 1971, Roland Madden and Paul Julian stumbled upon a 40 to 50 day oscillation when analyzing zonal wind anomalies in the tropical Pacific. The MJO is now characterized by an eastward progression of large regions of both enhanced and suppressed tropical rainfall that is observed over the Indian and Pacific Oceans.

Figure 1 illustrates the movement of the MJO in both boreal winter and boreal summer. The anomalous rainfall, shown in red, is usually first apparent over the western Indian Ocean and remains evident as it propagates over the warm tropical waters of the western and central Pacific. The rainfall then generally becomes zonal as it moves over the cooler ocean waters of the eastern Pacific but reappears over the tropical Atlantic and Indian Oceans. Each cycle lasts approximately 30 to 60 days and can be split into the wet and dry phases.

The phases of the MJO involve convection, which is the internal movement of currents within fluids. In the case of the Earth’s atmosphere, solar radiation heats the Earth’s surface, and this heat is then transferred to the air. A layer of air expands as it warms, becomes less dense, and is pushed upward by its buoyancy. It then cools and sinks to create a continuous cyclic air flow that influences local weather. In each MJO cycle, the wet phase is characterized first by enhanced convection and precipitation. It is then followed by a dry phase in which convection is suppressed.

Little attention was paid to this oscillation until a devastating El Niño event in 1982, after which scientists began to probe its global significance. Since then, MJO-related frequency variations in the tropics, both intra-annually and inter-annually, have been studied thoroughly. The MJO is now recognized as a significant contributor to the global climate and plays an important role in seasonal and inter-annual climate variations.
The resulting datasets contain averaged U (zonal, or latitudinal) and V (meridional, or longitudinal) wind velocities at pressure levels of 100, 200, and 300 mbar between 1979 and 1992. Pendent ozone data sets obtained by TOMS were calculated similarly.

The horizontal wind vector can be expressed as \( \mathbf{u} = \mathbf{u}_x \mathbf{i} + \mathbf{u}_y \mathbf{j} \) with \( \mathbf{i}, \mathbf{j} \) as the basis vectors of the longitude-latitude height coordinate system often used in atmospheric modeling. The Haimforth theorem, which states that any sufficiently smooth and rapidly decaying vector field can be resolved into irrotational (i.e., whose curl vanishes) and solenoidal (i.e., with divergence zero) component vector fields, can then be used to separate it into two scalar terms as where \( \mathbf{u} = \mathbf{v}_r + \mathbf{v}_s \) is the stream function (parameter of two-dimensional non-divergent flow whose value is constant along a streamline) and \( \mathbf{v} \) is the velocity potential (a scalar function whose gradient equals the vector velocity of an irrotational flow). Using these equations with the previously calculated (UV) pendent data, we have computed new (V, x) pendent datasets.

The annual cycle was then calculated and smoothed with a 30-day running mean. In the geosciences, an annual cycle is the part of a measured quantity’s fluctuation that is attributed to Earth’s changing position in orbit over the course of the year. The annual cycle is estimated from observational data by taking the average of all Januarys, all Februarys, and so forth. If the observational record is long enough and conditions are stationary, we can model a meaningful annual cycle that can be used to calculate an anomaly time series. The running mean of a time-series \( y(t) \) is calculated by finding the mean of all the \( y \) values in a neighborhood of \( x \), as shown by the formula

\[
\bar{y}_x = \frac{1}{N} \sum_{n=-N/2}^{N/2} y(x+n)
\]

where \( N \) is the size of the neighborhood. In the case of the annual cycle, the neighborhood is 30 days. This is done in order to smooth the function at the point \( x \). Pendent anomalies were obtained by removing the annual cycle from the pendent data. MJO anomalies were isolated through the difference between 15-day and 45-day running means of the pendent anomalies. This was done with a band-pass filter, or rejecting variability outside the range 30–90 days, so that only MJO-related variations (30–60 days) remained as fluctuations in the data.

In order to obtain a representative MJO vertical structure and spatial-temporal evolution, we used rainfall data from Global Precipitation Climatology Project (GPCP). MJO events were chosen based on an extended empirical orthogonal function (EOF) analysis, using a temporal lag of 11 pentads (from 5 to 5 pentads), of the GPCP rainfall MJO anomaly for the equatorial Indian Ocean and west Pacific (10°S–30°N, 130°E–150°W) and for the Northern Hemispheres “Wintertime”: November-April. The method of empirical orthogonal function (EOF) analysis is a decomposition of a signal or data set in terms of orthogonal basis functions that are determined from the data. The spatial-temporal patterns of the 1st EOF mode of GPCP rainfall MJO anomaly for boreal winter and boreal summer are depicted respectively in Figures 1a and 1b.

**INTRASEASONAL VARIABILITY AND MJO DEVELOPMENT**

Interest in tropical intraseasonal variability (TISV) has intensified in recent years, particularly the effect it has upon other climatic processes. Numerous global weather patterns have been carefully studied, including global catastrophes such as the Asian-Australian monsoon, El Niño, and La Niña. TISV appears to have an intricate relationship with the MJO, and studies indicate that the oscillation may cause significant fluctuations in the atmospheric concentrations of ozone and aerosols.

Ozone prevents UV radiation from reaching the Earth’s surface and is a key factor in global climate. Recently, the significance of ozone in the tropics has been recognized because of its oxidizing efficiency and contribution to the greenhouse effect. Various efforts have made an attempt to study the spatial distribution and temporal variation of ozone. This study investigates the intraseasonal variations of the total amount of tropical ozone and its connection to the MJO using data from the NCEP/National Centers for Environmental Prediction and TOMS.

Aerosols, the other important atmospheric compounds that are tracked, play a large role in cloud nucleation because the roughness of the available surface area on each particle promotes condensation. Furthermore, since clouds are central to the global movement of water, aerosols are easily transported between local climates. Concentrations of aerosols over the tropical eastern hemispheres are considerably variable in regions that are also strongly influenced by the MJO. Using TOMS aerosol data, we attempt to characterize the variability of aerosols and correlate our findings with MJO intraseasonal patterns.

**ANALYZING NCEP AND TOMS DATA**

Because the original datasets obtained from NCEP contain daily data for wind velocities, they have been averaged over 5-day periods, or pentads, for further analysis.
qualitatively inferred from Figures 2 and 3. The stream function used in this analysis can be interpreted as a description of the structure of upper-tropospheric cyclones and anticyclones. A (anti) cyclone is an area of low (high) atmospheric pressure characterized by inward (outward) spiraling winds that rotate counter clockwise in the Northern (Southern) hemisphere and clockwise in the Southern (Northern) hemisphere of the Earth. We are led to suspect that it is the MJO (anti) cyclones that affect the total ozone.

**INTRASEASONAL AEROSOL VARIABILITY**

The TOMS aerosol data was analyzed in a manner similar to the NCEP wind and TOMS ozone data. However, as aerosol indices can be both positive and negative, the absolute values of the daily data were used in calculated-pentadal averages. Selection of MJO events was separated into boreal winter (November-April) and boreal summer (May-September) in order to account for the seasonal differences in MJO variability as well as background circulation and aerosol loading. The spatial-temporal patterns of the 1st EOF mode of GPCP rainfall MJO anomaly for the boreal winter and boreal summer are depicted in Figure 1. The spatial-temporal patterns of the 1st EOF mode of the aerosol MJO anomalies for boreal winter and boreal summer are depicted in Figure 4. A brief look at Figure 4 suggests a very strong link between the aerosol and MJO rainfall variability. In general, over the Indian and western Pacific Oceans where the MJO convective-rainfall activity is strong, there is a very strong inverse linear relationship between aerosol content and rainfall. The regions of decreased aerosol content are shown in blue and overlap with the regions of increased MJO rainfall indicated by the solid black line. Similarly, the regions of increased aerosol content correspond to the regions of decreased rainfall. This is true for both boreal summer and winter. A more detailed study also demonstrates that the speed of the aerosol anomaly pattern exactly matches the speed of the MJO convection propagation, which confirms our findings.

**CONCLUSIONS**

We observed that the intraseasonal variations of total O₃ are considerable. It is evident that positive off-equatorial O₃ anomalies lead east of the equatorial MJO convection and negative off-equatorial O₃ anomalies lag west of the equatorial MJO convection. More detailed analyses show that this pattern propagates eastward in the Eastern Hemisphere at a phase speed similar to the MJO convection anomaly. Further analysis based on the dynamic fields demonstrates the similarity of the MJO patterns of total O₃ content and stream function. This in turn leads us to believe that the spatial pattern of the total O₃ anomaly is consistent with off-equatorial cyclones in the upper troposphere ahead of the MJO convection and with off-equatorial anticyclones following the MJO convection.

We have also observed a significant intraseasonal variability of aerosols and a clear connection of this variability with the MJO convection. As regions of suppressed aerosol index correspond to regions of enhanced precipitation, we hypothesize that the MJO washes out aerosols from the atmosphere with rainfall. The results of this study may enhance our predictive abilities by improving our knowledge of the tropical atmosphere. Given that the potential predictability of the MJO extends to lead times of 2 to 4 weeks, the strong connection between the intraseasonal variations of total O₃ aerosols, and the MJO convection implies that total O₃ variations and air quality may also be predicted with similar lead times over much of the tropics.

And, with improved prediction capabilities, perhaps the horrifying natural disasters that have recently been covered extensively by the media will soon become events of the past.

**ACKNOWLEDGMENTS**

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**FURTHER READING**


Imagine what it would be like if medical analysis and treatments could be performed internally by a single chip, if within seconds patients would know whether they have a dysfunctional kidney or a hyperactive thyroid. What if one day, instead of samples sent to outside labs for analysis of high cholesterol and immune deficiencies, and results not arriving for another couple weeks, a single drop of blood was enough to immediately determine any problems? What if just a small amount of saliva was enough to determine genetic predispositions to Alzheimer’s disease or Huntington’s disease?

Current developing technology has propelled an advanced medical frontier for diagnostic and treatment techniques. Microfluidics, an area of study that has emerged only recently in the 1990s, deals with precise control and manipulation of microscale and nanoscale volumes of fluids. Used in many areas such as DNA chips, where arrays of DNA representing genes are stored on a chip; micro-propulsion; micro-thermal technologies; and lab-on-a-chip technology, which involves a tiny chip that can perform multiple lab functions such as PCR, assays, and detection of abnormalities, this new research looks promising.

THE PROBLEM IN THE PAST

Although the proposed applications of microfluidics are exciting, the technology has been challenged on multiple fronts by other disciplines. In the past decade soft polymers such as poly(dimethylsiloxane) (PDMS) have emerged as the material of choice for microfluidic and soft lithography applications. PDMS offers the advantages of being affordable and simple to fabricate using rapid prototyping. It exhibits elastomeric properties with a surface energy of ~20erg/cm$^2$ and low Young’s modulus value of ~760kPa, allowing the material to easily conform and seal to other surfaces both reversibly and irreversibly. In addition, PDMS is optically transparent in the UV-visible light region, making UV and visible light useful for simple monitoring of fluid flows. Despite the advantages of PDMS in microfluidic applications, one of the most prominent drawbacks is its incompatibility with most organic solvents, including acyclic and cyclic hydrocarbons (pentanes, hexanes, heptane, cyclohexane), aromatic hydrocarbons (xylene, toluene, benzene), halogenated compounds (chloroform, trichloroethylene), ethers (diethyl ether, dimethoxymethane, tetrahydrofuran), and amines (diisopropylamine, dipropylamine, triethylamine). These solvents can cause swelling in the material, leading to changes in the cross-sectional area of microchannels and thus changing the rate of flow. The altered shape can then in turn affect surface properties and cause the device to unseal. Because of this, applications involve...
ing organic solvents require the use of other materials to construct the microfluidic device. Fluorinated compounds are more chemically robust and hence difficult to alter, but it is nearly impossible to construct microfluidic valves out of them by multilayer soft lithography (a process where a soft polymer is cast on a mold).

**THE CHosen SOLUTION**
Numerous methods have previously been utilized to create 3D structures. One of the most common techniques is known as the “membrane sandwich” method. This procedure involves the fabrication of three-dimensional systems by curing (hardening) multiple layers of single elastomers together. However, this method is limited by the difficulty of making effective interconnections between the layers during aligning, stacking, and bonding of patterned membranes during fabrication.

An alternative technique for producing such systems is the non-photolithographic method of solid-object printing. By emulating this technique, 3D wax molds could be printed without the need for masks or alignment, and they required only a computer file to direct the wax printer. A chemically robust perfused elastomer known as Sfil was used to create a solvent-resistant system [Figure 1].

Similar studies have used photocurable perfluoropoly-ethers to fabricate microfluidic devices. However, our particular design eliminates the intracacies involved with adhering partially cured perfluoropolyether layers. Since both the control layer and flow layer are formed in the same monolithic mold, this technique enables the use of elastomers that do not satisfy the adhesion requirements of multi-layer fabrication. After curing with heat, Sfil becomes a high-performance elastomer with extraordinary levels of resistance to chemicals, oil, and heat, allowing for a vast array of potential microfluidic applications in organic chemistry. With our new three-dimensional (3-D) valve design, we can fabricate microfluidic devices using fluorinated compounds in a single monolithic layer. Next, the design and development of 3-D microfluidic valves by molding Sfil onto printed wax molds encapsulated by PDMS is discussed.

**THE CREATION**
The wax molds were designed three-dimensionally in the program SolidWorks, and the file was printed directly onto a flat substrate using the commercial wax printing system SolidScape T66. Printing was carried out with two waxes: the building wax, which formed the structure of the desired microchannels, and the supporting wax, which acted as a sacrificial material and supported suspended structures during fabrication. The supporting wax was removed by placing the molds in a petri dish containing V3-O precision chaser for approximately one hour at 65°C. Afterwards the wax molds were cured at 40°C for approximately three hours. Once the molds were dry, twenty-five gauge pins were soldered into each valve. The PDMS blocks were created by pouring a liquid PDMS pre-polymer (mixture of 1:10 base polymer to curing agent ratio) onto a petri dish and allowing the mixture to cure at 70°C for one hour. Holes were punched through the PDMS and these PDMS blocks were then placed around each wax mold. Next, Sfil was poured over the molds and allowed to cure at 60°C for approximately twenty-four hours. Finally the building wax was melted away at 180°C to provide the completed structure.

**TESTING AND TUNING**
Figure 2 shows the three-dimensional pneumatic valves with a suspended fluid flow tube we designed, surrounded by a doughnut shaped pressure chamber. In this design, pressure was applied around the entire fluid channel, deflecting a thin Sfil membrane inward. This deflection closed the suspended channel and stopped fluid flow. Valve actuation was controlled by varying the pressure applied to the control channel.

As a result, these valves can be used for microfluidic metering and flow control. Introduction of fluid into this device was accomplished through inserting steel pins into the holes formed through the material. Sfil allows the formation of a tight seal around the input pins, readily accepting pressures up to 21 psi without leakage. In order to prevent malformations and to decrease the risk of pinholes in the fluid channel, the thickness of the tube needed to be at least 400μm. Four samples of adhesives (Sfil 610, Sfil 611, Sfil X-71-6030, and Sfil X-71-6054) were cured to milky white elastomeric materials and tested for adhesion to glass surfaces. Since fluid flow was pressure-driven, it was necessary to form an irreversible seal between the glass and Sfil. Both Sfil 610 and Sfil 611 adhered very strongly to glass substrates upon curing. However, the two materials were incredibly viscous which made it difficult to completely de-gas and remove all air bubbles. Sfil X-71-6054 was less viscous but peeled off from the glass too easily. Sfil X-71-6030 adhered well to the glass and was considerably less viscous than the other materials, which made it ideal for pouring over the wax molds. Because Sfil is one of the few liquid castable perfluoroesiloxanes available, further investigation may prove fruitful.

**DRAwBACKS**
However, this technique for fabricating Sfil-based microfluidic valves was time-consuming, expensive, and difficult to miniaturize due to restrictions in material properties. In addition, Sfil is opaque to UV-visible light, meaning it is incompatible with conventional optical methods of detection. Some of these limitations result from the properties of the material being used for the devices, whereas others are fabrication or design limitations. The performance of these microfluidic valves is not ideal, but as device complexity increases, similar design rules will evolve to yield the necessary high performance.

**CONCLUDING NOTES**
By replacing PDMS with Sfil, this novel three-dimensional wax printing method allows for making topological complex 3D microfluidic structures and offers many improvements over multi-layer soft lithography. These include ease of fabrication, rapid response time, and higher levels of integration. The major advantage of developing devices from 3D molding is that it enables the use of silicone elastomers that are more solvent-resistant than PDMS. Microfluidic devices constructed from Sfil provide many of the same advantages of PDMS with the
This technology makes the fabrication of more complex structures possible...

Table 1: Solubility parameters and swelling ratios of various solvents used in organic synthesis. ‘%’ in units of cal·cm⁻³·m⁻¹·K⁻¹ denotes the swelling ratio that was measured experimentally. $\delta$ indicates ‘$\delta$’ values, where $\delta$ is the length of PDMS in the solvent and D0 is the length of the dry PDMS. (Copyright from American Chemical Society, 2003.)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\delta^*$</th>
<th>$\delta$</th>
</tr>
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<tbody>
<tr>
<td>Pentane</td>
<td>7.1</td>
<td>1.44</td>
</tr>
<tr>
<td>Diisopropylamine</td>
<td>7.3</td>
<td>2.13</td>
</tr>
<tr>
<td>Hexanes</td>
<td>7.3</td>
<td>1.35</td>
</tr>
<tr>
<td>Triethylamine</td>
<td>7.5</td>
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<td>Ether</td>
<td>7.5</td>
<td>1.38</td>
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<tr>
<td>Cyclohexane</td>
<td>8.2</td>
<td>1.33</td>
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<td>Xylenes</td>
<td>8.9</td>
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<tr>
<td>Toluene</td>
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<td>Benzene</td>
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<td>Chloroform</td>
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</tr>
<tr>
<td>Methylene chloride</td>
<td>9.9</td>
<td>1.22</td>
</tr>
</tbody>
</table>

added advantage of chemical resistance. Since Sifel consists of a perfluoropolyether backbone, the elastomer is particularly stable due to the strength of the carbon-fluorine bond as well as the resulting steric hindrance arising from the strong forces between hydrogen and fluorine atoms. As a result, these chemically robust elastomers have the potential of increasing the number of solutions available for device applications. Because PDMS is incompatible with a broad range of solvents as reported by Lee et al., future work will involve the analysis of solvent compatibility of the listed organic solvents (with swelling ratios greater than 1.20) with Sifel (Table 1).

This technology makes the fabrication of more complex structures possible and has the potential to expand the field of highly integrated microfluidics to many new applications in chemical synthesis and analysis. In fact, maybe the next time you go to the doctor, your prescription will be a list of microfluidic devices.

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FURTHER READING
Spintronics, or spin-based electronics, is a relatively new area of research, involving the creation and evolution of electronic devices based not only on the charge, but also the spin of the electron. Electron spin is a property analogous to the angular momentum of a spinning ball of charge and as such has a magnetic dipole moment associated with it. The interaction of this spin with magnetic fields (applied externally or inherent in a material) is the underlying mechanism of spintronics devices. The appeal of these devices is the possibility of increased speed, decreased power consumption, and higher integration densities when compared to current silicon semiconductor technology.

The magnitude of an atom’s magnetic dipole moment is the net sum of the dipole moments of its electrons; in most atoms these dipole moments cancel each other out. In a ferromagnetic (FM) material, however, unpaired outer shell electrons give each atom a permanent net dipole moment, and these moments interact and align parallel to one another in regions called magnetic domains. Magnetization of the ferromagnetic material occurs when it is placed in an external magnetic field strong enough to cause all domains to align parallel to the externally applied field. At this point, the material’s inherent magnetic field will tend to polarize the spin direction of carrier electrons transported through the material.

The giant magnetoresistance effect (GMR), first reported in 1988, occurs in structures consisting of alternating thin film layers of ferromagnetic and nonmagnetic materials. When current passes through one ferromagnetic layer, it undergoes spin polarization, causing the spins of the electrons in the current to align parallel to the magnetic field of the material. Once the current has been polarized, the resistance of additional ferromagnetic layers will depend on the alignment of the magnetic field of each layer relative to the polarization of the current. When the magnetic field orientation of the ferromagnetic layers are parallel, the resistance of the structure is at a minimum. When they are antiparallel, more spin-dependent scattering will occur as the polarized
electrons attempt to pass into the material aligned against their polarization, and resistance is at a maximum. When the nonmagnetic “spacer” layers between the ferromagnetic layers are very thin and consist of insulating material, the tunnel magnetoresistance effect is observed, in which the transport mechanism between ferromagnetic layers is tunneling rather than conduction.

Currently, the most widely used spintronics device is the spin valve, whose applications include read-write heads on hard disks and magnetoresistive random access memory, or MRAM. A spin valve consists of two ferromagnetic layers sandwiching a thin layer of nonmagnetic material, typically a nonmagnetic metal. It is designed such that at least one of its FM layers has a controllable magnetic alignment. For instance, the two FM layers can each possess a different coercivity, a quantity related to the intensity of externally-applied magnetic field required to annul or reverse the material’s magnetic alignment. The “hard” layer has a higher coercivity and is therefore more resistant to realignment of its magnetic field, whereas the “soft” layer has a lower coercivity and will reverse its magnetic alignment at a lower magnetic field magnitude. A present challenge in the field of spintronics is the integration of these devices into existing semiconductor technology. Advantages of the use of semiconductors, and particularly organic semiconductors (OEs), in spintronics include their economical large-scale fabrication, tunable electronic properties, and long spin diffusion length (this affects the average distance electrons travel in the material before losing their spin polarization). The use of an OOE as the middle “spacer” layer of a spin valve has been demonstrated; however, these layers often contain defects such as pinholes, which are small holes penetrating down into the layer. When evaporation is used to deposit the top FM layer of a spin valve, these defects can allow the evaporated metal to penetrate into the lower layer, occasionally creating shorts that allow electron flow to completely bypass spacer layers. Xiong et al. determined that in an organic semiconductor spacer layer deposited via evaporation, these defects extended at most 100nm into the layer, and thus the effective spacer layer depth was the actual depth minus 100nm. In this project, we seek to eliminate this problem without necessitating thick spacer layers. The soft lithography technique of microcontact printing (μCP) utilizes an elastomeric “stamp” to transfer a thin film layer or pattern onto a substrate [Figure 1]. Deposition of the top FM layer of a spin valve via printing from an elastomeric stamp will ideally prevent the ferromagnetic material from penetrating into the defects of the spacer layer, eliminating shorts in the device.
This technique was demonstrated using a self-assembled monolayer (SAM) of 1,8-octadecanethiol (H2CH2CH7SH) as an initial spacer layer, with 1-octadecanethiol (CH3(CH2)17SH) used initially to demonstrate monolayer deposition. The strong interaction between the Au and the thiol endgroups induces the formation of a densely packed, oriented monolayer structure of alkane-thiol oligomers adsorbed onto the Au surface. The dominant electron transport mechanism through alkane-thiol monolayers is tunneling. Although a SAM does not form pinhole defects, it can be used in place of an organic semiconductor for the purpose of demonstrating microcontact printing.

Monolayer Deposition

During the first stage of fabrication, a monolayer of alkane-thiol was deposited on the surface of an Au thin film via self-assembly. Two types of substrates were used for deposition of the self-assembled monolayer (SAM): an Au thin film evaporated directly onto a glass substrate and a Si wafer with 2900 Å SiO2 coated with a 25 Å Cr adhesion layer and 500 Å of Au via evaporation. These were cleared into chips of approximately 0.25 cm² area. The samples were immersed in a solution of 10 mM 1-octadecanethiol (Sigma-Aldrich) or 10 mM 1,9-octadecanethiol (Alfa Aesar) in ethanol for at least 10 hours, then rinsed with ethanol and blown dry with N2. Samples were then tested using various techniques to verify deposition of a SAM on the surface of the Au. An image of 1-octadecanethiol deposited onto an Au thin film on a glass substrate is displayed in Figure 2. While an initial resistance in the 10 MΩ range was observed for this monolayer (as opposed to 20 Ω for the underlying Au thin film), rapid oxidation of the SAM in air, particularly on the uneven surface of the glass/Au substrate, prevented thorough electrical characterization of the sample.

A poly(dimethylsiloxane) (PDMS) elastomeric stamp was fabricated using Sylgard 184 (Dow Corning) and cleaned for 15 seconds with O₂ plasma. A 200 Å Au thin film layer was then deposited on the surface using evaporation. Before thin film transfer, the PDMS stamp was rinsed with ethanol and dried using N₂ gas. The stamp was then placed across half of the monolayer surface and light, smoothing pressure was applied to ensure conformal contact. The surfaces were left in contact for a few minutes before the stamp was removed. The other half of the monolayer surface was left bare.

While attempts to transfer a thin Au film to the surface of an alkane-thiol monolayer using microcontact printing were unsuccessful, printing onto an alkane-thiol surface produced complete transfer of the thin film from the surface of the PDMS stamp to the alkane-thiol monolayer, as was confirmed visually. As opposed to alkane-thiol, alkanedithiol molecules possess thiol groups on each end, allowing the thiol and groups of the monolayer to form solid bonds with the Au surfaces both above and below the monolayer. These bonds enable the Au thin film to overcome adhesion forces to the PDMS stamp so that it could transfer neatly from the stamp to the monolayer. Figure 3 contains atomic force microscopy (AFM) images of the surface of the sample at the edge of the transferred Au thin film. Both optical and APM images confirmed the existence of cracks and wrinkles in the film, although 1-V (current-voltage) measurements confirmed that the film was electrically continuous.
Future Work

Before proceeding with device fabrication, optimization of the microcontact printing technique may include adjusting the \( \text{O}_2 \) plasma cleaning conditions and duration, the use of a metallic wetting layer between the PDMS and the Au film to increase film smoothness, and/or the use of a rigid backplane for the elastomeric stamp to decrease cracks and wrinkles in the thin film. A master pattern will then be created using photolithography, and from this a patterned elastomeric stamp will be cast using PDMS and replica molding, according to the following procedure:

1. A thin film of photoresist is spin-coated onto a wafer.
2. A pattern (photomask) is created using a printed transparency (initially) or using e-beam lithography (eventually).
3. The photoresist is exposed to light through the photomask, hardening the exposed areas.
4. The remaining photoresist is developed, leaving a master pattern consisting of the hardened areas of photoresist.
5. An elastomeric stamp is cast using replica molding:
   a. PDMS precursor is poured over the master pattern.
   b. The PDMS is cured and the stamp is peeled off of the master pattern.
6. The PDMS stamp will be cleaned with \( \text{O}_2 \) plasma and an Au thin film will be immediately evaporated onto its surface.

Microcontact printing will then be performed as described above, and the device will be tested to ensure that a complete, continuous, smooth pattern transfer has taken place. Possible refinements at this stage may include adjustment of the layer thicknesses and/or tailoring of the PDMS rigidity to achieve smooth and precise pattern transfer onto the substrate.

The next stage will involve the evaporation of a Co thin film onto the stamp, sandwiched between two thin Au layers. These layers are necessary to prevent the Co from reacting with the PDMS and/or the air. Once this step is successfully completed, fabrication and testing of a complete spin valve device will be attempted:

1. Wire of ferromagnetic material sandwiched between two Au thin film layers will be evaporated onto a Si wafer using evaporation with a shadow mask.
2. A SAM of alkanedithiol will be deposited onto the Au surface.
3. The PDMS elastomeric stamp, coated with Au/Co/Au, will be stamped across the top of the structure. The device will be thoroughly tested and characterized and the fabrication procedure will be appropriately adjusted to achieve optimal device performance.

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Further Reading

Probing Slow Folding of Cytochrome c’

It is absolutely necessary for proteins to be synthesized and folded in living cells accurately and efficiently. Mistakes in the folding of proteins lead to a myriad of disorders, including Alzheimer’s disease, cystic fibrosis, and Mad Cow Disease, just to name a few. Misfolded proteins tend to accumulate in the cell, and without the ability to degrade them, the cell dies. Understanding the folding mechanism of proteins is the first step towards treatments for these serious disorders. As such, one of the greatest challenges facing modern chemistry and biology is predicting how a protein folds based on its amino acid sequence. This is otherwise known as the protein folding problem. The production of biologically functional proteins requires the newly synthesized polypeptide string of amino acids to find a uniquely stable conformation from a vast array of possible folds. Recent advances have established the “energy landscape” theory, in which protein folding follows specific pathways involving the formation of partially structured intermediates. A variety of folding mechanisms have so far been postulated, but the factors affecting such pathways and the folding kinetics of many proteins remain unknown, and are still subjects of intense investigations.

Cytochrome c’ is a Model Protein

The protein cytochrome c’ from Rhodopseudomonas palustris (cyt c’) is an excellent model system for investigating gating factors. A soluble, 125-residue protein, cyt c’ is a four-helix bundle that functions as an electron carrier in its bacterial host. Cyt c’ contains a prosthetic heme group covalently linked to cysteine residues at positions 113 and 116 [Figure 1]. Cyt c’ exhibits highly heterogeneous folding. It includes fast-folding populations folding on a millisecond timescale in addition to slow-folding populations folding on the order of minutes. This phenomenon is particularly interesting because helical bundle structures are inherently fast folding, as evidenced in previously the studied proteins cyt b₆₅₃ and acyl-CoA binding protein.

What Causes Slow Folding?

This investigation is primarily focused on understanding the factors that contribute to slow folding kinetics of cyt c’. Specifically, we aim to investigate the dependence of cyt c’ folding kinetics on final denaturant concentration, pH and protein concentration, which tells us the role of driving force, misfolding and protein aggregation in the folding process, respectively. In addition, the slow-folding species in a number of proteins, including the closely related iso-2 cyt c, are produced by proline cis-trans isomerization [Eq. 1]. Since cyt c’ contains four proline residues, it is reasonable to speculate that proline cis-trans isomerization may also be responsible for the slow folding phase of cyt c’. We have attempted to answer this question by using cyclophilin (CyP), a peptidyl prolyl cis-trans isomerase which catalyzes the isomerization reaction, and observing its effect on the folding kinetics of cyt c’. Moreover, we would also like to investigate the correlation between slow folding kinetics and aggregation propensity of cyt c’. Deposition of structured protein aggregates has been associated with a variety of pathological conditions, such as the amyloid fibrils in Alzheimer’s and Parkinson’s diseases. Previous studies have shown that intermolecular aggregate formation slows down the folding kinetics of the closely related cyt c’, and we would like to test if a similar effect operates in our case.

We also aim to characterize the equilibrium unfolding of a variant of cyt c’, L12H, by monitoring its responses towards thermal and chemical denaturant. This gives us information about the stability of the mutant and guides future kinetic studies on related mutants using fluorescent energy transfer
UNFOLDING CYT C’ AT EQUILIBRIUM

The proportions of cyt c’ unfolded at various guanidine hydrochloride (GuHCl) concentrations between 0-6 M were monitored with absorption, CD and fluorescence emission spectroscopy (graphs not shown). The midpoint of the sigmoid curves obtained indicates the relative stability of cyt c’. Two different pH conditions were explored, pH5 and pH7, since the folded and unfolded protein are structurally different at the two pHs. The folded species has 50% bis-His-ligated heme at pH5 but this reaches 100% at pH7. Comparatively, the unfolded species has no bis-His-ligated heme at pH5 but 75% at pH7. The midpoint of titration curves from all three spectroscopies is consistently 1.5 M GuHCl, and no significant difference is observed between curves constructed at these two pHs. This shows that the mutant cyt c’ is unstable relatively to the wildtype protein. In addition, the experiment was repeated using an unfolded stock of protein. The signal of the unfolded protein is the same as that of the folded at high GuHCl concentrations, but at low GuHCl concentrations it is only 75% of that of the folded, meaning only 75% of the unfolded protein refolds back to its native conformation.

SLOW FOLDING KINETICS OF CYT C’

To measure the dependence of cyt c’ folding kinetics on GuHCl concentration, four concentrations ranging from 0.3 M to 1.2 M were used, at both pH5 and pH7 [Figure 3]. In the difference spectra (insets in Figure 3) between the first and last data point, the spectral peak at pH7 becomes more distinct with higher GuHCl concentration, whereas at pH5 the traces for all GuHCl concentrations almost overlap on each other. The decay in absorbance is much faster at lower GuHCl concentration than at higher concentration, indicating that the rate of refolding decreases with increasing GuHCl concentration. However, the effect of denaturant was much less pronounced at pH5 than at pH7.

In addition, folding kinetics of cyt c’ were also measured at three different protein concentrations, ranging from 2.2 μM to 34 μM, at 0.3 and 1.2 M GuHCl [Figure 4]. We have found that the change in absorbance is much slower at lower protein concentrations (2.2 μM and 6.7 μM cyt c’) at both GuHCl concentrations. At high protein concentration (34 μM cyt c’), however, there is a very rapid decrease in absorbance during the initial few seconds, as evidenced by the fact that the kinetic decay traces are almost flat lines. However, this decrease occurred during the initial mixing time and was not captured in the experiment.

EXAMINING PROLINE CIS-TRANS ISOMERIZATION

We first overexpressed cyclophilin in E. coli and purified it with ion exchange and affinity chromatography. The activity of purified CyP was assayed [Figure 5A], and the values of kcat/Km for each experiment were plotted as a function of CyP concentration [Figure 5B]. The slope of the line gives the kcat/Km value of CyP activity, which is 3.6 × 10̅⁸ M⁻¹s⁻¹ at 15 °C. This is equivalent to 5.0 × 10̅⁸ M⁻¹s⁻¹ at 20 °C, assuming that the Arrhenius pre-exponential factor Qe = 2. In comparison with the previously reported value of 2.0 × 10̅⁸ M⁻¹s⁻¹ at 20 °C, our CyP is only fractionally active. Although this was less than ideal, the problem was overcome by using four times as much CyP in our kinetic experiments.

The refolding kinetics experiments of cyt c’ described above was then repeated in the presence of 20 μM CyP at two GuHCl concentrations (Fig. 6). However, no significant acceleration of the refold-
ing kinetics was observed in the presence of CyP at both GuHCl concentrations.

RATIONALIZING SLOW FOLDING
The stability of L12H cyt c' from all three methods is similar at pH 5 and 7, indicating that the pre-formation of His-heme native contact in cyt c' does not influence its stability, produced possibly by the delicate balance of various interactions, including hydrogen and ionic bonds and hydrophobic interaction, that exist in the protein structure. The comparison between the titration curves of folded and unfolded cyt c' shows that folding is only 75% reversible, whereas the remaining 25% is permanently misfolded during the process. Misfolded cyt c' can produce aggregates that may affect its folding kinetics, which will be discussed later. The consistency of the results generated by each of these three spectroscopies indicates that the folding-unfolding transition occur at the same denaturant level for each structural feature studied, global as well as local. The refolding kinetics of cyt c' was slower at higher final GuHCl concentration, because the higher the GuHCl concentration results in a more denaturing environment. Thus, the free energy difference between the folded and unfolded states is reduced, producing a smaller driving force for folding. This shows that the thermodynamic driving force has a direct correlation to folding kinetics. However, such denaturant effect was much less pronounced at lower pH levels. This might be due to the effect of misalignment by other residues such as lysine or N-termminus. Moreover, the disappearance of the slow phase of absorbance change during refolding at higher protein concentration, i.e. in environment which promotes protein aggregate formation, suggests that such absorbance changes may be contributed by the aggregation of misfolded protein rather than the intrinsically slow kinetics of cyt c' folding. This observation suggests an alternative pathway in addition to those described above, in which misfolded cyt c' produces aggregated structures that are unable to refold to the native conformation.

However, CyP did not accelerate the folding of cyt c', thereby indicating that the slow folding of cyt c' is not due to proline cis-trans isomerization. This result is in stark contrast with many other proteins, including iso-2 cyt c. Though surprising, it may be attributed to the fact that all four proline residues in cyt c' adopt the trans conformation in the folded state, which is four times more likely than the cis conformation in most proteins. Thus the folding process only requires the conversion of a small fraction of cis-proline to its trans isomer, and therefore is less dependent on the process of proline cis-trans isomerization. Other processes, such as the aggregation of misfolded protein, may be the rate-determining step.

WRAPPING UP
This experiment showed that increasing final denaturant concentrations increase the rate of absorbance change during folding, while lower pH diminishes the effect of the denaturant. Aggregation of misfolded protein also partially contributes to the slow absorbance change during refolding of QIA cyt c'. Moreover, proline cis-trans isomerization does not contribute to the slow folding phase of cyt c'. Future work may include the fitting of folding kinetics data to single-exponential decay curves, so that exact values of rate constants could be obtained for more quantitative comparison. The effect of preformed native contact and the refolding reversibility of the pseudo-wild type cyt c' need also be explored.

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FURTHER READINGS
Paris Hilton, Jesus Christ, Tazmanian Tigers, and Islamic Extremism are all elements of a simple graphite line, no thicker than a grain of rice. The line contains all of the experiences that have affected me over the passage of time. Through the use of line, narrative and iconography, I explore human constructions concerning faith, time, and history. I attempt to provoke the viewer to participate in my personal observance of human response to everyday life.

Growing up in the California Central Valley, I was consistently surrounded by agriculture, nature, and classic cars. I attempted to capture the fascination around me through the medium of drawing, and soon realized the experiences attached to me like barnacles to a whale. In the same way a whale creates a current, as it travels through the sea, every line effects a drawing. Every new image contains more lessons, ideas, and wisdom than its predecessor. Therefore, drawing inflicts a conviction of having to keep learning about and experiencing significant things.

The work presently displayed is a collection inspired by the mass media’s self-sustainability. I think its both interesting and tragic, the way people are transformed into icons, then boasted on by the same group that helped distinguish them. Like the rest of the world, I observe this destruction and am forced to react to it based on what I know.

**Eric Nyquist**

Eric is graduate of Art Center College of Design, Eric is a working artist in Los Angeles, California. More of his work can also be seen in:

- Communication Art Illustration Annual (July 2007)
- CMYK Issue #38 (August 2007)
- ericnyquist.com
- artfoodemywalls.com

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1. "Construction", Serigraph, 14x17"
2. "Tunnel", Mixed media, 33x20"
3. "Subsistence", Mixed media, 5x7"
4. "Prey", Mixed media, 22x30"